# Obesity and Insulin Resistance in Human Growth Hormone Transgenic Rats[1]

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## Abstract

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A line of transgenic rats (heterozygotes) carrying a chimeric gene comprising a regulatory portion of murine whey acidic protein and a structural portion of human GH (hGH) genes developed severe obesity with age. To characterize physiological mechanisms that lead to fat accumulation, an array of parameters related to obesity were studied. Blood hGH levels were continuously low, endogenous rat GH secretion was suppressed, and the pulsatility in peripheral GH levels was absent. Plasma glucose, insulin, triglyceride, and FFA levels in the male transgenic rats significantly exceeded those in nontransgenic littermates at 12 and 17 weeks, but not at 7 weeks, of age. All symptoms except hyperlipidemia were restored to normal by treatment with an antidiabetic agent, thiazolidinedione (troglitazone), for 1 week from 17 weeks of age. As phenotypic expression of obesity was already evident before aberration of physiological parameters, it was assumed that animals had a condition in which obesity or hyperlipidemia caused hyperinsulinemia. Gene expression and enzymatic activity of lipoprotein lipase in the adipose tissue in the transgenic rats were not different from those in normal rats. In contrast, the gene expression level of glycerol-3-phosphodehydrogenase was markedly elevated, suggesting that glycerol synthesis was much enhanced in the adipocytes of the transgenic rats. In an ip glucose tolerance test, the transgenic rats were not hyperglycemic at 7 weeks of age; however, the animal became hyperglycemic at 15–17 weeks of age. Finally, treatment with recombinant hGH for 1 week to produce pulsatile secretion reduced the size of epididymal and kidney fat pads and restored normal weight gain. These observations suggest that continuously low peripheral GH levels with the lack of pulsatile secretion resulted in obesity and noninsulin-dependent diabetes mellitus.

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GH AFFECTS carbohydrate as well as lipid metabolism (1). The majority of the effects is believed to originate in enhanced production of insulin-like growth factor I (IGF-I) by hepatocytes (2). Some direct effects of GH on

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carbohydrate and lipid metabolism in the adipose tissue have been proposed (3). No simple and straightforward explanations for understanding the GH action on carbohydrate and lipid metabolism are presently available. Moreover, the signal pathways for GH and insulin seem to converge at postreceptor levels, although they clearly differ at the receptor level (4, 5). GH is secreted in a pulsatile manner that displays gender specificity in mature animals (6, 7, 8), and this secretory pattern appears to play a role in the regulation of body growth and metabolic activities of liver and adipose tissues.

Investigations of transgenic mice secreting foreign GH in extrapituitary sites have contributed to the understanding of the effects of chronic exposure of high levels of GH (9, 10, 11, 12, 13). We have produced two lines of transgenic rats from two different founders, integrating different copy numbers of the transgene (14); one line displayed relatively higher levels of serum human (h) GH, extended body length, and increased weight gain (high line), whereas the other had relatively lower levels of serum hGH and normal body length, but was severely obese (low line). Reproductive phenotypes in these lines of transgenic female rats were completely different from those in normal females (15). Due to a decrease in the number of somatotrophs, endogenous GH secretion in both lines of transgenic rats was inhibited. Unlike serum hGH levels in high line rats, serum hGH levels in low line rats were low (14). These low line animals developed symptoms characteristic of obesity and insulin resistance around 12 weeks of age.

Therefore, in the present study, the development of these symptoms was studied by measuring plasma glucose, insulin, triglyceride, and FFA levels 5 weeks before and 5 weeks after the onset of insulin resistance. The adipose tissue of these transgenic rats was characterized by assessing gene expression of lipoprotein lipase and glycerol-3-phosphodehydrogenase (G3PDH) at 17 weeks of age. In addition, plasma tumor necrosis factor-$\alpha$ (TNF$\alpha$) levels were also measured. Insulin resistance was further confirmed by glucose tolerance tests and treatment with an antidiabetic agent, thiazolidinedione. Finally, at 17 weeks of age, these transgenic rats were treated with recombinant hGH for 1 week to evaluate the importance of pulsatile GH secretion for diabetogenic events in metabolism.

# Materials and Methods

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### Animals

Generation of the low line transgenic rats have been described previously (14). They were housed in a room at 23 C with a lighting schedule of 14 h of light and 10 h of darkness (lights on at 0500 h). The low line transgenic male (a heterozygote) was mated with normal adult female rats, and the resulting transgenic and nontransgenic male littermates were used. All transgenic offsprings displayed uniform phenotypes, probably due to the high linkage between the transgenes integrated.

Four or five each of the male transgenic rats and their nontransgenic male littermates were killed at 4, 7, 12, and 17 weeks of age; body weight, nose to tail length, and weight of the epididymal fat pad were recorded. Another group of male transgenic rats (four to eight rats each) and their littermates were killed

at 7, 12, and 17 weeks of age, and plasma glucose, insulin, triglyceride, and FFA were measured with commercial kits (Wako, Tokyo). Plasma insulin was determined using a commercial RIA kit (Amersham Life Science, Tokyo, Japan).

**Analysis of the volume and number of epididymal fat cells**

Adipose tissue was obtained from the epididymal fat pad of the transgenic and control rats or their nontransgenic littermates at 17 weeks of age, fixed in 10% neutral buffered formalin, dehydrated in a graded series of alcohol solutions, and embedded in paraffin. The tissues were cut sagittally into 6-μm thick sections. Measurements of adipose tissue volume, cell size, and number of cells were based on the methods reported by Sasaki *et al.* (16).

**Troglitazone treatment and blood sampling**

The male transgenic rats and control rats at 17 weeks of age were treated for 1 week with troglitazone (an analog of thiazolidinedione, Sankyo Co., Tokyo, Japan) at a concentration that results in approximately 150 mg/kg·day. To accustom them to the handling procedures, animals were carefully patted daily. On the final day of treatment, the tip of the tail was cut using a razor blade while animals were conscious, and oozing blood was drawn into four or five hematocrit tubes (50 μl). The tubes were centrifuged, and the plasma was collected and stored at -20 C until assayed for glucose, insulin, triglyceride, and FFA.

**The ip glucose tolerance test**

Male transgenic and control rats at 7 and 15–17 weeks of age were fasted overnight for 12 h and received an ip injection of 0.5 g/kg BW D-glucose (20% solution). Blood samples were obtained from the tail vein without anesthesia, and glucose concentrations were determined before (0 min) and 15, 30, 60, 90, 120, and 150 min after the glucose injection.

**Preparation of complementary DNA (cDNA) probes for lipoprotein lipase (LPL) and G3PDH**

cDNA probes were synthesized by RT-PCR using the following oligonucleotide primers: LPL forward primer (5'-CGCGCTCTAGTCCTCTGACG-3'; rat LPL cDNA; nucleotides 64–83) and LPL reverse primer (5'-TTCTTCCTCCAGCCAGTTGA-3'; nucleotides 590–571), G3PDH forward primer (5'-TGAAGGTCGGTGTCAACGGATTTGGC-3'; rat G3PDH cDNA; nucleotides 35–60) and G3PDH reverse primer (5'-CATGTAGGCCATGAGGTCCACCAC-3'; nucleotides 1017–994).

Total RNA was extracted from the adipose tissues of mature intact male rats by the method described previously (17). In brief, 1 μg total RNA was incubated at 42 C for 60 min in a final volume of 20 μl with 50 U cloned Moloney murine leukemia virus reverse transcriptase (Gene AmpTM RNA PCR kit, Perkin-Elmer, Norwalk, CT). After reaction, 20 μl of the RT mix were added to 100 μl of the PCR solution (containing 5 U AmpliTaq DNA polymerase) and each of the forward and reverse primers (50 pmol each). The PCR conditions were as follows: denaturation, annealing, and elongation at 94, 60, and 72 C, respectively, for 1 min each for 35 cycles, followed by a 10-min final extension at 72 C. PCR products were analyzed by electrophoresis on a 1% agarose Tris-acetate EDTA (buffer) gel. cDNA fragments of LPL and G3PDH were ligated into T7Blue T-vector (Novagen, Madison, WI), transformed in NovaBlue strain *Escherichia coli*, cloned, and proliferated. The plasmid DNA was recovered from

each transformant. The insert of each clone was sequenced, and nucleotide sequences were confirmed. For hybridization probes, *Eco*RI/*Pst*I fragments of each clone were purified and radiolabeled using the random primed method.

**Northern hybridization for LPL and G3PDH**

An aliquot (10 μg) of the total RNAs from each transgenic or control male rat at 17 weeks of age was denatured at 65 C for 15 min and electrophoresed in a 1% 3-(*N*-morpholino)propanesulfonic acid (MOPS)-formaldehyde agarose gel, and the bands of rRNAs were detected by ethidium bromide staining (2 μg/ml). After washing with distilled water, the agarose gel was photographed, and the RNA was transferred to a nylon membrane (Biodyne B Membrane, Pall BioSupport, Port Washington, NY). The membranes were prehybridized with salmon sperm DNA and then hybridized with the specific probes described above. The membrane washed once with 2 x SSC (standard saline citrate)-0.1% SDS for 10 min at room temperature, twice with 0.5 x SSC-0.1% SDS for 5 min at 45 C, and then twice with 0.1 x SSC-0.1% SDS for 5 min at 45 C. The transfer membrane was exposed to x-ray film (Eastman Kodak, Rochester, NY) for 72 h at -70 C. The pictures of ribosomal RNA (rRNA) and autoradiographs were scanned (GT-6500, Epson, Tokyo, Japan), and the relative intensity of each band was analyzed using NIH imaging software.

**LPL activity and TNFα concentration**

Total LPL activity in the fat pad of transgenic or control male rats at 17 weeks of age was measured as previously described (18). Plasma TNFα concentrations in transgenic or control male littermates (n = 5) at 12 weeks of age were measured by ELISA with a commercial kit (BioSource International, Camarillo, CA). The standard curve for TNFα concentrations was linear between 2.3–150 pg/ml.

**hGH treatment**

One group of 12-week-old transgenic rats was injected ip with recombinant hGH (Pharmacia, Uppsala, Sweden) at a dose of 100 μg/rat·0.3 ml vehicle, four times daily at 4-h intervals between 0700–1900 h for 7 days. Another group of 12-week-old transgenic and control rats was treated with vehicle only for 7 days. Changes in the plasma hGH concentration after the first single injection of hGH or vehicle to transgenic rats were determined by RIA. The mean daily body weight gain during the 7-day treatment period was monitored. Tissue samples were collected 3 h after the final injection, and weights of the liver, epididymal fat pad, and kidney fat pad were recorded.

**Statistical analysis**

The data presented in Tables 1–4, and Figs. 2–4 were analyzed by ANOVA followed by Student's *t* test. The data presented in Fig. 6 were analyzed by ANOVA followed by Duncan's multiple range test. In all statistical tests, the difference was considered significant at $P < 0.05$.

**View this table:** [in this window] [in a new window] **Table 1.** Changes in body weight and nose to tail length with age in transgenic rats and nontransgenic littermates

**View this table:** [in this window] [in a new window] **Table 2.** Cell area and cell number in the epididymal fat pad of transgenic rats and nontransgenic littermates at 17 weeks of age

**View this table:** [in this window] [in a new window] **Table 3.** Changes in plasma glucose, insulin, triglyceride, FFA, and TNFα in transgenic rats and nontransgenic littermates

**View this table:** [in this window] [in a new window] **Table 4.** The effects of troglitazone treatment (150 mg/kg·day) for 1 week from 17 weeks of age on carbohydrate and lipid metabolism in transgenic rats

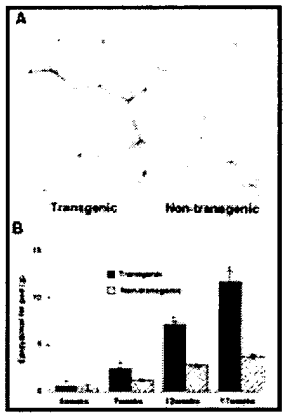


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**Figure 2.** Histology of the epididymal fat at 17 weeks of age (A) and changes in tissue weight with age (B). Values are the mean ± SEM of each four rats. *, Different from normal rats (nontransgenic littermates), $P < 0.01$.

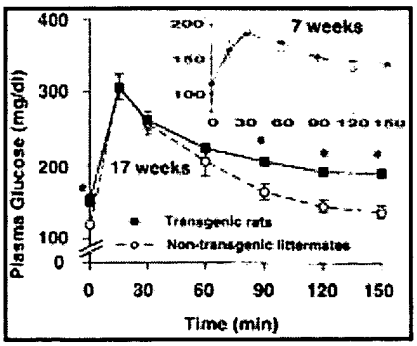


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**Figure 3.** Intraperitoneal glucose tolerance test in the transgenic and normal rats (nontransgenic littermates) at 7 (*inset*) and 15–17 weeks of age. Each *curve* represents the results from four or five male rats. Values are the mean ± SEM. *, Different from normal rats (nontransgenic littermates), $P < 0.05$.

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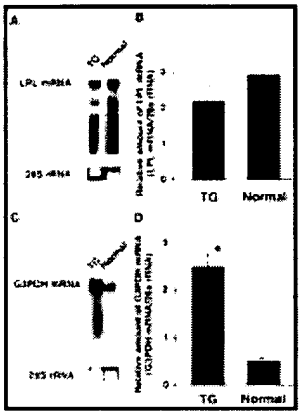


**Figure 4.** LPL and G3PDH mRNA levels in the epididymal fat pad at 17 weeks of age in the transgenic (TG) and normal (nontransgenic littermates) rats. A, A representative Northern blot of LPL mRNA. B, Densitometric quantification of Northern blot for LPL mRNA levels. C, A representative Northern blot of G3PDH mRNA. D, Densitometric quantification of Northern blot for G3PDH mRNA. Values are the mean ± SEM of each three independent analyses.

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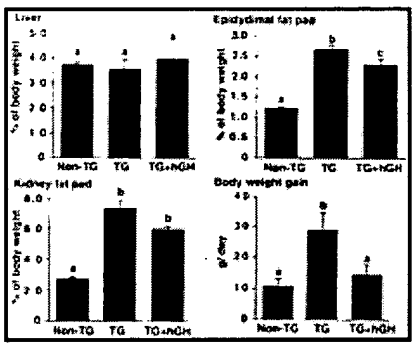


**Figure 6.** Relative weights of liver, epididymal fat pad, and kidney fat pad at the end of repetitive hGH injections for 1 week and average body weight gain per day during the treatment. The former three parameters are expressed as a percentage of body weight. Non-TG, Nontransgenic littermates without the treatment; TG, transgenic rats without the treatment; TG+hGH, transgenic rats treated with hGH as described in *Materials and Methods* and in Fig. 5. Values are the mean ± SEM. Values with different superscript are significantly different ($P < 0.05$).

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# Results

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**Low line hGH transgenic rats develop massive adipose tissue**

Male low line transgenic rats had a much larger abdominal girth at 25 weeks of age (Fig. 1), although they had the same head to tail length as their nontransgenic littermates (Table 1). Body weights of the transgenic rats did not differ from those of the controls at 7 or 12 weeks of age, but increased thereafter and were significantly heavier than those of the controls by 17 weeks of age (Table 1). The

higher rate of body weight gain due to fat accumulation continued through 25 weeks of age, as shown in Fig. 1.

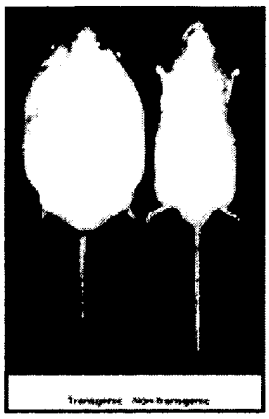

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**Figure 1.** Representative features of a hGH transgenic rat (*left*; 1.2 kg BW) and its normal nontransgenic littermates (*right*; 560 g BW) at 25 weeks of age. Note that nose to tail lengths are similar in both individuals.

The area of epididymal adipocytes in histological specimens taken at 17 weeks of age was much larger in the transgenic rats than that in the controls (Fig. 2A). Growth of the epididymal fat pad between 4–17 weeks was much faster in the transgenic rats (Fig. 2B). The average size of adipocytes at 17 weeks of age (Table 2) was approximately 3 times that in the controls and was calculated to be 5.6-fold in volume larger in the transgenic rats. The numbers of cells per adipose tissue pad were similar in the two groups.

**Plasma concentrations of lipids and TNFα, and glucose-insulin homeostasis**

At 7 weeks of age, plasma glucose and insulin levels in the male transgenic rats were not different from those in their nontransgenic littermates. At 12 weeks of age, however, the transgenic animals had significantly higher glucose and insulin levels. Plasma triglyceride and FFA levels in the transgenic rats were also higher than those in their nontransgenic littermates, but FFA levels at 7 weeks of age were not significantly different. Plasma TNFα levels in the transgenic rats at 12 weeks of age were lower than those in controls (Table 3).

The results of an ip glucose tolerance test are presented in Fig. 3. After a 12-h fast, the transgenic rats at 7 weeks of age were not hyperglycemic (Fig. 3, *inset*), but at 15–17 weeks of age, transgenic rats were modestly, but significantly, hyperglycemic compared with their littermates. In the transgenic rats 90, 120, and 150 min after an ip glucose load, elevated plasma glucose remained at a significantly higher level than that in the nontransgenic controls.

**LPL activity and its gene expression, and G3PDH gene expression in adipose tissue**

LPL activity per whole epididymal fat pad activity in the transgenic rats (729 ± 31 U/tissue) was also not significantly different from that in their nontransgenic littermates (812 ± 42 U/tissue). LPL gene

expression in the adipose tissue of the male transgenic rats or their nontransgenic littermates at 17 weeks of age was quantified by Northern hybridization (Fig. 4A). LPL gene expression in the transgenic rats was not significantly different from that in normal rats when expressed after normalization with quantified rRNA (Fig. 4B).

G3PDH gene expression in the adipose tissue of the male transgenic or their nontransgenic littermates at 17 weeks of age was quantified by Northern hybridization (Fig. 4, C and D). G3PDH gene expression in the male transgenic rats was about 5-fold higher than that in their nontransgenic littermates when expressed after normalization with quantified rRNA.

**Effects of troglitazone on glucose and lipid metabolism**

The male transgenic rats were treated with troglitazone (an antidiabetic agent enhancing postinsulin receptor signal transduction) for 1 week from 17 weeks of age. Plasma glucose (nonfasting state), insulin, triglyceride, and FFA concentrations were determined at the end of the treatment and are summarized in Table 4. Plasma glucose, insulin, and triglyceride concentrations decreased significantly by 16%, 36%, and 71%, respectively, whereas only FFA levels increased significantly by 51%.

**Effects of repeated hGH injections on the male transgenic rats**

The male transgenic rats were treated repeatedly with recombinant hGH to simulate pulsatile changes in peripheral GH concentrations. The experimental schedule and a peripheral hGH profile after the first single injection are shown in Fig. 5, A and B. A single ip injection of hGH at a dose of 100 μg/rat resulted in a peak serum hGH level of approximately 120 ng/ml at 60–90 min after the injection; the concentration returned to basal levels within 3–4 h.

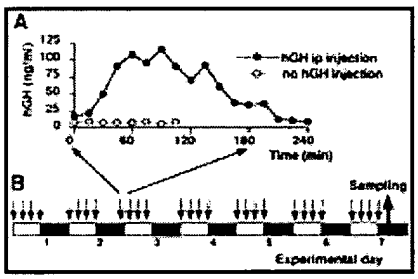


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**Figure 5.** A, A representative pattern of serum hGH after the first single ip injection of hGH of the day. B, Experimental schedule of hGH injections. hGH was injected four times a day between 0700–1900 h (light phase) for 7 days. *White* and *black bars* represent light and dark phases, respectively. *Arrows* represent ip hGH injection (100 μg/0.3 ml $H_2O$).

Under the influence of pulsatile hGH level simulation, the relative weights of the epididymal fat pad and the kidney fat pad decreased in the male transgenic rats (Fig. 6). On the other hand, the treatment slightly increased the relative weight of the liver, but the increase was not significant. The treatment significantly reduced 1-week body weight gain in the male transgenic rats, rendering it comparable to that in nontransgenic littermates (Fig. 6), although daily food intake did not significantly change (data not presented).

# Discussion

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As was described in previous reports (14, 15), the endogenous rGH in the transgenic rats in this study was almost completely suppressed. Immunohistochemical analysis of the anterior pituitary in these animals indicated that the number of somatotrophs largely decreased (data not presented). Low endogenous GH levels are due at least in part to the marked decrease in somatotroph population. Moreover, the WAP promoter integrated in the expressing vector in this study kept peripheral hGH concentrations at low levels (~10 ng/ml plasma) throughout the day (14). Thus, peripheral GH concentrations in these transgenic rats remained low during the experimental period. Nonetheless, somatic growth in the male transgenic rats, as assessed by nose to tail length and liver weight, occurred in an entirely normal way. This indicated that the low GH levels in the peripheral circulation were sufficient to support normal somatic growth. Various strains of GH transgenic mice have been established and their phenotypes reported. With a few exceptions, a marked increase in body size (nose to tail length) has been reported (19, 20). The difference in body size among GH transgenic animals seems to result from the difference in peripheral levels of both endogenous and foreign GH.

The GH gene, in contrast to other genes, linked to the whey acidic protein promoter in transgenic mice leads to expression in tissues other than the mammary gland, particularly in brain glial cells and to a limited extent in liver (22, 23). If this were also the case in the rat transgenic model, then ectopic expression of hGH in the brain would lead to high levels of pituitary exposure and result in inhibition of somatotroph development, as observed in this study. Thus, in addition to GH, the profiles of IGF and IGF-binding protein levels in cerebrospinal fluid need to be evaluated to obtain deeper insight into various phenotypes expressed in these obese transgenic rats.

It is well documented that obesity is frequently associated with hyperinsulinemia, hyperglycemia, and/or hyperlipidemia, the typical symptoms of insulin resistance (21, 24, 25). The transgenic rats in this study deposited excess body fat as they grew and manifested all of these symptoms together with impaired glucose tolerance. It is a matter of controversy whether obesity is the cause or the result of insulin resistance (26, 27). The order of the emergence of these symptoms offers an important clue (25). In this study, the significant fat deposition in the epididymal fat pad was already evident at 4 weeks of age, whereas the onset of hyperinsulinemia, hyperglycemia, and hyperlipidemia was delayed until 12 weeks of age. Thus, the present results suggest that obesity is the cause rather than the result of insulin resistance.

Troglitazone, an analog of thiazolidinedione, given orally has been shown to decrease blood insulin, glucose, triglyceride, and FFA concentrations in various insulin-resistant and obese animal models, such as the KKAy mouse, the obese (*ob/ob*) mouse, and the Zucker fatty rat (28). Recently, analogs of thiazolidinedione have been intensively studied and shown to bind to the peroxizome proliferator activator receptor, inducing adipocyte differentiation and enhancing transcription of many genes, including an *ob* (leptin) gene (29, 30, 31). Although *ob/ob* mice express a mutant leptin molecule,

troglitazone is still effective in ameliorating the insulin-resistant symptoms; therefore, leptin is not the only gene product responsible for symptoms of insulin resistance. As troglitazone treatment improved most of the symptoms of insulin resistance in the transgenic rats used here, it appears that a lack of insulin responsiveness was the cause of the symptoms.

Troglitazone given genetically obese animals has been shown to decrease blood FFA (28). In those animals, it appears that an elevation of FFA is due to insulin insensitivity of the adipose tissue, and thus, troglitazone is effective in restoring normal FFA levels. In the present study, however, troglitazone was totally ineffective in decreasing elevated FFA levels. Thus, it appears that the origin of high plasma FFA levels in the transgenic rats was not related to insulin resistance and hence differs basically from that in other mutant animal models for obesity. In obese Zucker rats, fat accumulation is thought to be mainly attributable to an elevated LPL activity in the adipose tissue (32). Recent reports indicate that TNF$\alpha$ production from adipocytes is increased in those mutant animal models, which at least in part causes insulin resistance (33, 34, 35). However, gene expression and enzymatic activity of LPL in the adipose tissue from low line rats in this study were not different from those in the normal rats, and blood TNF$\alpha$ levels in the transgenic rats at 12 weeks, the age at which hyperinsulinemia became evident, were lower than those in normal rats. Thus, TNF$\alpha$ did not play a role in the symptoms of insulin resistance, suggesting that insulin resistance in the transgenic rats must be different from that in other experimental models.

It has been suggested that FFA, glycerol concentrations, and their turnovers are proportional to an increase in the adipose tissue mass without deteriorating glucose tolerance, and that high FFA induces insulin resistance in liver and muscle (26). Thus, it is also possible that an elevation of FFA levels in the transgenic rats may not result from insulin resistance of the adipose tissue, but it may be the cause of obesity. In addition, the level of G3PDH messenger RNA (mRNA) has previously been used as a marker of insulin sensitivity, as G3PDH mRNA levels in cultured neonatal rat adipocytes are regulated by insulin (36). In the adipose tissue from the transgenic rats at 17 weeks of age, G3PDH gene expression was strongly enhanced, but it may be premature to conclude that the adipose tissue in the transgenic rats was sensitive to insulin. It is not clear at present whether G3PDH gene expression can be used as an *in vivo* marker for insulin susceptibility of adipocytes in adult animals. It is possible that other factors may also affect G3PDH gene expression in adopocytes *in vivo*.

The pulsatile and sexually differentiated secretory patterns of GH observed in mature animals in various species are believed to be important for regulating growth and metabolic functions of liver and adipose tissue (6, 7, 8). A relationship between GH secretory pattern and obesity has been described in humans and rodents; GH treatment in GH-deficient patients alters body composition (37, 38, 39), dietary obesity is induced in the female GH-deficient dwarf rat (38), and monosodium glutamate-treated rats gain weight when peripheral GH concentrations are low (40, 41, 42). In the view of these observations, the results of this study suggest that obesity in hGH transgenic rats could have resulted from low GH secretion. This is further supported by the experiment in which the transgenic rats were treated with recombinant hGH intermittently for 1 week. This decreased adipose tissue weight as well as body weight gain during the treatment period without changing food intake. Thus, the treatment must have

suppressed fat synthesis but enhanced utilization of accumulated fat.

It is well documented that GH-deficient human adults show obesity and insulin resistance (27). The insulin resistance transgenic animal model described here could provide a useful experimental basis for developing diagnostic and therapeutic approaches to noninsulin-dependent diabetes. A disease condition where insulin resistance has not propagated to the adipose tissue can be ranked as an intermediate status, followed by final progression into a wasting noninsulin-dependent diabetic status. Based on the results of the present investigation, this intermediate status can be diagnosed with high FFA levels insensitive to troglitazone treatment and normal TNF$\alpha$ levels. This situation could possibly be improved by treatment with appropriate amounts of GH.

In summary, by using the hGH transgenic rats, it was demonstrated that low GH levels devoid of pulsatile secretion in the peripheral circulation are sufficient to support normal body growth, yet can induce a type of obesity that is responsive to simulation of pulsatile GH secretion. Further investigation is required to elucidate the molecular mechanism of this obesity induction and progression.